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(71)(72) Applicants and Inventors: LISZIEWICZ, Julianna [DE/US]; 4400 East West Highway, Bethesda, MD 20814 (US). LORI, Franco [IT/US]; 4400 East West Highway, Bethesda, MD 20814 (US).

(74) Agent: LOOPER, Valerie, E.; 11726 Lightfall Court, Columbia, MD 21044 (US).

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(57) Abstract

A method for treating HIV infection in human beings comprising the steps of measuring viremia, and if viremia is less than 50,000–100,000 copies per milliliter, administering a combination of compounds selected from the group consisting of hydroxyurea, and one or more reverse transcriptase inhibitors.

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COMPOSITIONS FOR INHIBITING HUMAN IMMUNODEFICIENCY VIRUS USING HYDROXYUREA AND A REVERSE TRANSCRIPTASE INHIBITOR

Field of the Invention

The present invention relates generally to the field of treatment of human beings with reverse transcriptase dependent viruses such as Human Immunodeficiency Virus (HIV) infections. The inventors have found that the combination of hydroxyurea (HU) and a reverse transcriptase inhibitor without a protease inhibitor can reduce the level in the blood to non-detectability (less than 500 copies per milliliter) and can also be used for long-term therapy (years) in human beings without provoking viral rebound, even in cases where patients have developed genotypic resistance to the reverse transcriptase inhibitor. This combination is relatively inexpensive, well-tolerated, and forgiving of irregularities in the medication regimen.

Background of the Invention

The inventors had previously disclosed the first known case where a patient who had been HIV positive stopped treatment without immediate rebound of the viral population in the blood. In USSN 08/812,515, filed March 7, 1997, Method of Inhibiting Human Immunodeficiency Virus by combined use of Hydroxyurea, a nucleoside analog, and a protease inhibitor, which is incorporated by reference as if set forth in full, it was disclosed that a triple drug combination including hydroxyurea, a reverse transcriptase inhibitor, and a protease inhibitor, can drive the virus to undetectable levels in both the blood, and more significantly, the lymph nodes, according to the most sensitive tests then available. At the time, it was supposed that the triple combination derived much of its potency from the protease inhibitor.

As recently as a year ago, drug cocktails which did not use hydroxyurea such as a triple drug combination involving the use of AZT, 3TC and protease inhibitors had been suggested for the treatment of HIV-1 infection and

eradication of the virus. The efficacy of this combination was thought to originate from the potency of the protease inhibitors and the mechanism of action of the AZT/3TC combination in inhibiting the rebound of resistant mutants. And indeed, many patients experienced impressive drops in viral load in the bloodstream, so that virus was undetectable, with virus becoming undetectable in blood for some patients in as little as eight to sixteen weeks. Viral load, measured as HIV-1 RNA, is the best available indicator of disease progression and reduced concentration of HIV-1 in various tissues and fluids in response to antiretroviral therapy, and is predictive of improved prognosis (Mellors, J.W. et al. Science 272(5265) 1167-1170, 1996). Viral load in the blood is more conveniently determined than viral load in other tissues.

Despite their promise, protease inhibitors are new drugs which must be explored in detail before they are marketed. As a result, they are expensive enough to be impractical for many patients. Most protease inhibitors must be taken on a very exacting schedule, or they will lose their effect. The drugs do not always elicit a patient response (defined as a significant drop in plasma viral load). Neither the protease inhibitors nor 3TC easily penetrate to certain organs such as lymph nodes and the brain, and the combination of protease inhibitor, AZT and 3TC apparently does not completely eradicate HIV-1 in macrophages or in quiescent cells, which are major reservoirs of HIV-1. Further, patients who have interrupted therapy using AZT, 3TC and protease inhibitors and then rebounded cannot be as effectively treated with either the same combination or the same combination with another protease inhibitor because they develop resistant mutants. Many times the resistance to one protease inhibitor extends to others. There currently exists a pool of patients who, having used a protease inhibitor in the past, can no longer benefit from the newer protease inhibitors. Finally, the protease inhibitor-containing combinations without hydroxyurea have shown at best, response rates of 80-90% and "failure" - a combined figure including people who never responded to therapy, those who could not tolerate side effects, those who responded initially but later saw a return of detectable virus, and those who had difficulty adhering to the strict dosing regimens required by the drugs. See Project

Inform Perspective 23:1-3, November 1997.

One explanation for the eventual rebound of viral load after apparently successful treatment may be found in Identification of a Reservoirs for HIV-1 in Patients on Highly Active Retroviral Therapy, Finzi, et al., Science Magazine 278 (5341):1295. In a study of 22 patients successfully treated with highly active anti-retroviral therapy for up to 30 months, a highly aggressive new test method found replication-competent virus was routinely recovered from resting CD4+ T lymphocytes. The frequency of resting CD4+T cells harboring latent HIV-1 was low, 0.2 to 16.4 per million cells, and in cross-sectional analysis, did not decrease with increasing time in therapy.

Hydroxyurea has been widely used over the last three decades for the treatment of leukemia, sickle cell anemia, and has more recently been suggested for use in the treatment of HIV infections. EPO patent publication 94918016.0 filed May 17, 1994 and corresponding to USSN 08/065,814, filed May 21, 1993, which is incorporated herein as if set forth in full, describes the *in vitro* data suggesting that it would be worthwhile to try administration of hydroxyurea in combination with ddI in human volunteers. The data shows that hydroxyurea used alone had an antiviral effect, and that ddI was the more potent of the two when each were used alone *in vitro*. When the combination was tried in human beings, a therapeutic effect was observed in that the viral load in the blood, or plasma viremia, was reduced , although the amount of virus in the blood never went below the threshhold level of detection. In one study Jessen et al. JAMA 277:18 1437-8 1997, hydroxyurea and ddI were used in combination for up to 65 weeks, but the patients did not reach an undetectable level (defined as less than 500 copies per ml).

In a later study, a therapeutic effect was again found, but the amount of virus in the blood failed to reach 100 copies per milliliter, the thresshold level of detection for the test used in that study. See Lori et a., Combination of a Drug Targeting the Cell with a Drug Targeting the Virus Controls Human Immunodeficiency Virus Type 1 Resistance, AIDS Research and Human Retroviruses, vol. 13, Number 16, Mary Ann Liebert, Inc. (1997). Further, patients who were treated with both hydroxyurea and the reverse transcriptase inhibitor ddI had higher rates of formation of ddI resistant mutations than those

treated with ddI alone, although the overall level of virus remained low and rather constant. An incident was reported where two individuals on hydroxyurea and ddI treatment for a year stopped treatment for a year, without rebound. However, the initial level of viral load for each of them was so low that others have doubted whether these individuals had an infection.

The inventors have now found that hydroxyurea in combination with a reverse transcriptase inhibitor alone can be used to reduce the level of viral load in the blood to undetectable levels (less than 500 copies per milliliter), and that such treatment can be sustained without rebound over long periods of time. This discovery yields an important tool, particularly for use in treating patients for whom treatment with protease inhibitors is impractical, but also in a generalized scheme of treatment for the disease.

Summary of the Invention

It is an object of the present invention to provide a method of inhibiting the replication of retroviruses such as HIV-1, HIV-2, HTLV-1 and HTLV-2 and other reverse-transcriptase-dependent viruses such as Hepatitis B virus in human cells. A further object of this invention is to provide a course of treatment for HIV infections that reduces the presence of the virus in both plasma and the lymphoid system, and which inhibits viral rebound after cessation of treatment. It is yet a further object of this invention to provide a method of treating HIV infection which includes an indication of the type of drug combination most advantageously used under different circumstances. Yet another object of this invention is to provide a treatment for HIV which relatively less expensive and has relatively low toxicity, therefore increasing its suitability for widespread use in a large population.

The present inventors have found that, where indicated, the combination of hydroxyurea (HU) and one or more reverse transcriptase inhibitors such as 2',3'-dideoxyinosine (ddI) and 2',3'-didehydro-2'3'-dideoxythimidine (d4T) may be used to achieve the same end result as the protease inhibitor combinations without hydroxyurea. These modes of treatment, in addition to the use of combinations including hydroxyurea, protease inhibitors, reverse transcriptase inhibitors, and other immunizatiion techniques, can be combined to yield an

method of treatment that is more effective, and minimizes both expense and toxicity to the individual.

These and other objects and advantages of the present invention will become apparent through the text and examples herein.

Brief Description of the Drawings

Figs. 1 and 1A compare the response of patients to ddI alone and ddI plus hydroxyurea. Fig. 1 shows virus levels in the blood over time, and Fig. 1A shows CD4 cell counts over the same time frame.

Fig. 2 describes data derived for thirty-six patients for a 40 week study using HU and ddI therapy.

Fig. 3 describes data derived from a follow-up on 12 patients, for an average of 28 months using HU and ddI therapy.

Figs. 4 and 4A compare the response of patients to combinations of ddI, d4T and a placebo with ddI, d4T, and hydroxyurea. Fig. 4 shows virus levels in the blood over time, and Fig. 4A shows CD4 cell count over time. A change occurs at the 12 week mark where patients "nonresponsive" to the placebo combination replaced the placebo with hydroxyurea.

Detailed Description of the Invention

Hydroxyurea is one of many inhibitors of ribonucleotide reductase, an enzyme known for catalyzing the reduction of ribonucleoside diphosphates to their deoxyribonucleoside counterparts for DNA synthesis. In the present invention, Hydroxyurea inhibits viral replication, and also acts to down-modulate the immune system. Another material which inhibits viral replication and down-modulates the immune system is cyclosporine, a cyclophilin inhibitor. Other ribonucleotide reductase inhibitors include guanazole, 3,4-dihydroxybenzo-hydroxamic acid, N,3,4,5-tetrahydroxybenzimidamide HCl, 3,4-dihydroxybenzamidoxime HCl, 5-hydroxy-2-formylpyridine thiosemicarbazones, and α-(N)-heterocyclic carboxaldehyde thiosemicarbazones, 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone, N-hydroxy-N'-amino-guanidine (HAG) derivatives, 5-methyl-4-aminoisoquinoline thiosemicarbazone, diaziquone,

doxorubicin, 2,3-dihydroxylbenzoyl-dipeptides and 3,4-dihydroxylbenzoyl-dipeptides, iron-complexed 2-acetylpyridine 5-[(2-chloroanilino)-thiocarbonyl]-thiocarbonohydrazone (348U87), iron-complexed 2-acetylpyridine-5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone (A1110U), 2'-deoxy-2'-methylenecytidine 5'-diphosphate (MdCDP) and 2'-deoxy-2', 2'-difluorocytidine 5'-diphospahte (dFdCDP), 2-chloro-9-(2-deoxy-2-fluoro-ß-D-arabinofuranosyl)-adenosine (Cl-F-ara-A), diethyldithiocarbamate (DDC), 2,2'-bipyridyl-6-carbothioamide, phosphonylmethyl ethers of acyclic nucleoside analogs, [eg. diphosphates of N-(S)-(3-hydroxy-2-phosphonylmethoxypropyl and N-2-phosphonylmethoxyethyl) derivatives of purine and pyrimidine bases], nitrosourea compounds, acylclonucleoside hydroxamic acids (e.g., N-hydroxy-α-(2-hydroxyethoxy)-1(2H)-pyrimidineacetamides 1-3, and 2-acetylpyridine 4-(2-morpholinoethyl)thio-semicarbazone (A723U)).

Hydroxyurea has been widely used in cancer therapy as a broad spectrum antineoplastic drug (R. C. Donehower, *Seminars in Oncology* 19 (Suppl. 9), 11 (1992)). Hydroxyurea is readily absorbed after oral ingestion, rapidly distributed in the body fluids, including the cerebrospinal fluid, and enters cells efficiently by passive diffusion (*Id.*). Its toxic effects are less profound and easier to control than other chemotherapeutic drugs (*Id.*).

In human chemotherapy, hydroxyurea is currently administered using two basic schedules: (a) a continuous daily oral dose of 20-40 mg per kg per day, or (b) an intermittent dose of 80 mg per kg per every third day. Either schedule could be used in the treatment of viral infections. Given the present invention, lower dosages of hydroxyurea may also be effective in treating HIV infections. The presently preferred dosage range for use of hydroxyurea in treating HIV infections is 800-1500 mg per day, which can be divided over a 24 hour period, for example as 300-500 mg three times a day (TID), assuming an adult weighing about 70 kg. When the patient's weight is over 60 kg, 400 mg TID is preferred, for those under 60 kg, 300 mg TID is preferred. Hydroxyurea is classified as a mildly toxic drug and does not cause immunosupression. Myelotoxicity is hydroxyurea's dose-dependent toxicity. However, such toxicity can be easily monitored and it is constantly and rapidly reversible after

decreasing the dose or suspending the treatment (Donehower, R.C., *Semin. Oncol.* **19**:11 (1992). By monitoring simple parameters such as neutrofils, platelets and red blood cell counts, hydroxyurea can be administered for years,and sometimes for decades.

A second member of the combination of the present invention is a reverse transcriptase inhibitor. Examples include nucleoside analogs, such as the 2',3'-dideoxyinosine (ddI)(available as Videx® from Bristol Myers-Squibb) used in the Examples. Nucleoside analogs are a class of compoounds known to inhibit HIV, and ddI is one of a handful of agents that have received formal approval in the United States for clinical use in the treatment of AIDS. See Clinical Microbiology Reviews, Supra, p. 200. Like zidovudine (3'-azido-2',3' -dideoxythymidine or azidothymidine [AZT] available from Glaxo Wellcome), zalcitabine (2',3' - dideoxycytidine [ddC](available as Hivid® from Hoffman-La Roche), lamivudine (2'-deoxy-3'-thiacytidine [3TC] available as Epivir® from Glaxo Wellcome) and stavudine (2',3' -didehydro-2',3'-dideoxythimidine [D4T](available as Zerit® from Bristol Myers-Squibb), ddI belongs to the class of compounds known as 2',3' - dideoxynucleoside analogs, which, with some exceptions such as 2',3'-dideoxyuridine [DDU], are known to inhibit HIV replication, but have not been reported to clear any individual of the virus. Other nucleoside reverse transcriptase inhibitors include adefovir (Preveon® a an adenine nucleotide analog from Gilead Sciences) abacavir (1592U89 available from Glaxo Wellcocme), and lubocavir (a guanosine analog available from Bristol Myers-Squibb). Non-nucleoside reverse transcription inhibitors include nevirapine (Viramune™ available from Boehringer Ingelheim Pharmaceuticals, Inc.), delaviridine (Rescriptor® available from Pharmacia & Upjohn) and efavirenz (available as Sustiva®, from DuPont Merck)

Currently, antiviral therapy requires doses of ddI at 200 mg BID or 400 mg once (QID) per day for an adult human. Similar dosages may be used in the present invention. However, use of the combination drugs may increase the effectiveness of these nucleoside analogs so that they can be used at lower dosages or less frequently. In combination with hydroxyurea, the presently preferred range for ddI is 100-300 mg twice a day (BID), assuming an adult

weighing 70 kg. When d4T is used with either hydroxyurea or a combination of hydroxyurea and ddI, the preferred range is 40 mg BID.

The inventors have found that, in certain cases, the same result achieved by triple and quadruple drug combinations including protease inhibitors can be achieved without using the protease inhibitor, and employing instead a combination of hydroxyurea and one or more revese transcriptase inhibitors. The present invention, also includes a method of analyzing whether the protease inhibitors are required, and selecting a treatment regimen. Thus this invention includes a course of treatment that, at one time or another, may include the use of one or more protease inhibitors.

Of the potential protease inhibitors, compounds such as hydroxyethylamine derivatives, hydroxyethylene derivatives, (hydroxyethyl)urea derivatives, norstantine derivatives, symmetric dihydroxyethylene derivatives, and other dihydroxyethylene derivatives have been suggested, along with protease inhibitors containing the dihydroxyethylene transition state isostere and its derivatives having various novel and high-affinity ligands at the P_2 position, including 3-tetrahydrofuran and pyran urethanes, cyclic sulfolanes and tetrahydrofuranylglucines, as well as the P_3 position, including pyrazine amides. In addition, constrained "reduced amide"-type inhibitors have been constructed in which three amino acid residues of the polypeptide chain were locked into a γ– turn conformation and designated γ-turn mimetics. Other alternatives include penicillin-derived compounds, non-peptide cyclic ureas. At present, the inventors have no basis for distinguishing among the many potential protease inhibitors that may be used in combination with HU and a reverse transcriptase inhibitor.

The protease inhibitors used in the present invention include Indinavir sulfate, (available as Crixivan™ capsules from Merck & Co., Inc, West Point, PA) saquinavir (Invirase® and Fortovase® available from Hoffman-LaRoche), ritonavir (Norvir® available from Abott Laboratories), ABT-378 (Abott Laboratories) and nelfinavir (Viracept® available from Agouron Pharmaceuticals), GW141 (available from Glaxo Wellcome/Vertex).

In addition to reverse transcriptase inhibitors and protease inhibitors, the present invention may utilise integrase inhibitors such as AR177 (Zintenvir® available from Aronex); fusion inhibitors such as pentafuside, (T-20) and cytokine inhibitors (available from Chiron) and chemokine inhibitors.

Suitable human dosages for these compounds can vary widely. However, such dosages can readily be determined by those of skill in the art. For example, dosages to adult humans of from about 0.1 mg to about 1 g or even 10 g are contemplated.

As disclosed in USSN 08/812,515, the combination of compounds of the present invention may be administered by any conventional route, and the particular dosage, toxicity, and mechanism for delivery of the individual of drugs of the present invention are either already known, or can be readily determined by conventional empirical techniques, as can dosages for the combinations. The use of combinations may result in the ability to use lower amounts of one or more of the constituents. One of ordinary skill in the art will recognize that different dosages and intervals may be appropriate.

The present invention is based on the recognition that there are multiple sources of new viral particles being produced during the course of the disease, that different drug combinations may used to control them, and that the amount of virus in the blood can be used as an indicator of which type of combination is most advantageously used.

Where very high viral loads in the blood indicate that certain types of activated T-cells are producing large amounts of viral particles, a combination including a protease inhibitor is indicated. Such a combination may also advantageously include hydroxyurea and one or more reverse transcriptase inhibitors, integrase inhibitors, fusion inhibitors and cytokine inhibitors. Particularly preferred are the reverse transcriptase inhibitors including AZT, 3TC, ddC, ddI, d4T, abacivir, adefovir, nevirapine, delviridine, efavirenz, and mixtures thereof. Of these, ddI and d4T and mixtures thereof are most preferred.

Where viral loads in the blood are lower, the activated cells are producing less virus, and the role of the quiescent cell begins to predominate. Then a combination targeting cells such as quiescent lymphocytes and

macrophages is indicated. Generally, where the level of virus in the blood is about 50,000 copies per mL or less, combinations including hydroxyurea and one or more reverse transcriptase inhibitors are preferred. Where virus in the blood has reached about 500 copies per mL, and especially when it is 200 copies per mL or less, such combinations are even more preferred. Particularly preferred for use with hydroxyurea are the reverse transcriptase inhibitors including AZT, 3TC, ddC, ddI, d4T, abacivir, adefovir, nevirapine, delviridine, efavirenz, and mixtures thereof. Of these, ddI and d4T and mixtures thereof are most preferred.

The drug combinations using hydroxyurea but not a protease inhibitor of the present invention may be used before and after acute infection, before seroconversion, and after seroconversion, as well as before and after various other types of treatment, so long as the plasma viral load is no more than about 50,000-100,000 copies per mL. Further, the hyroxyurea-containing combinations (without protease inhibitors) can administered prophylactically to high-risk individuals without raising concerns about the viability of protease inhibitor therapy for that individual in the future. Where viral load is no more than about 20,000 copies per mL, is preferred that the treatment be continued for at least about twelve to fifteen months. Depending on the status of the patient, the time of the treatment can be from several months to lifelong.

The present invention also contemplates the possibility of deliberately activating certain types of quiescent cells during combination therapy. As disclosed in Atty Docket No. 7002, activation of the immune system during treatment can be used to reduce the viral population harbored by quiescent cells, and may provide a therapeutic advantage. The cells can be activated by vaccination against any of a number of diseases known to activate such cells, including, for example, HIV-1, Hepatitis B, Influenza, and Polio vaccination. HIV-1 genetic immunization is preferred, as disclosed in USSN 60/604,627, filed February 21, 1996. Such activation should preferably take place after the elimination of active virus production (that is, after the patient's viral load is undetectable (defined as less than about 200 copies per mL) for at least 2 months). Repeated activation would be helpful to ensure that all quiescent cells harboring HIV-1 DNA had been activated.

The following Examples are presented for the purpose of illustrating the practice of the present invention. They do not limit the invention, or the claims which follow.

Example 1

Sixty HIV-infected subjects, who were asymptomatic and had about 250 to 500 CD4 cells per μl (a normal cell count) and who may have had previous treatment, but not with ddI, were divided into two groups. Group 1 had twenty patients with a dosage of 200 mg ddI twice daily. Group 2 had forty patients with a dosage of 200 mg ddI twice daily plus 500 mg hydroxyurea twice daily. The study duration was twenty-four weeks, with a possible extension to 48 weeks. Figure 1 shows the results for the two populations as variation in plasma viremia over time. The combination of ddI plus hydroxyurea worked better than ddI alone, although a slow downward trend was noticeable, virus was still detectable in the patients blood. In addition, the CD4 cell counts for those treated with the double combination were lower than those receiving only ddI. This raised some cause for concern, as HIV-1 infection typically causes a decline in both the numbers of CD4 cells and their effectiveness. See Fig. 1A

Example 2

The study continued with thirty-six patients on the combination of ddI and hydroxyurea. After forty weeks, the curve was either flat or beginning a slow rise.

Example 3

After 42 *months* all of the 12 follow-up patients had undetectable (less than 200 copies per milliliter) virus in the blood. Eleven out of twelve had reached undetectable status by about fifteen months. None had rebounded. The shape of the curve is not inconsistent with the hypothesis that a second source of viral particles predominated after about ten months.

Example 4

In another study, the combinations of hydroxyurea plus ddI and ddI plus d4T were compared. One group received 40 mg d4T twice daily, 200 mg ddI twice daily, and 500 mg hydroxyurea twice daily. The second group received 40 mg d4T twice daily, 200 mg ddI twice daily, and a placebo. At twelve weeks, 54% of the group receiving the hydroxyurea combination had fewer than 200 copies of virus per mL, compared to the placebo group. Those with less than 200 copies were then subjected to a more sensitive test, and 19% of the hydroxyurea group had less than twenty copies of virus per mL, while the placebo group had 8% with less than 20 copies of virus per mL. Non-responders (defined as those with more than 200 copies of virus per mL were present in both groups: the hydroxyurea group had 40% and the placebo group had 71%. At twelve weeks, the code of randomization was opened and patients were subsequently treated according to virological response. Thus, a number of patients formerly treated with a placebo were started on hydroxyurea as well, yielding a triple combination of ddI, d4T and hydroxyurea. All combinations showed a drop in virus levels, with the population possibly beginning to rise by the 48th week. Addition of hydroxyurea to the control group decrease virus level in the blood, and also decreased and then increased CD4 cell counts.

WE CLAIM:

1. An improved method of treating HIV infection in human beings, comprising the steps of :
testing the blood for viral load;
if the viral load is above about 50,000-100,000 copies per ml, administering a drug combination consisting of hydroxyurea, one or more reverse transcriptase inhibitors, and one or more protease inhibitors;
if the viral load is below about 50,000-100,000 copies per ml, administering a drug combination consisting of hydroxyurea and one or more reverse transcriptase inhibitors.

2. The method of Claim 1, wherein the reverse transcriptase inhibitor is selected from ddI, d4T, 3TC, AZT, delavirdine, abacavir, adefovir, nevirapine, efavirenz, lubocavir and mixtures thereof.

3. The method of Claim 1, wherein the protease inhibitor is selected from Indinavir, saquinavir, ritonavir, Nelfinavir, GW141, and mixtures thereof.

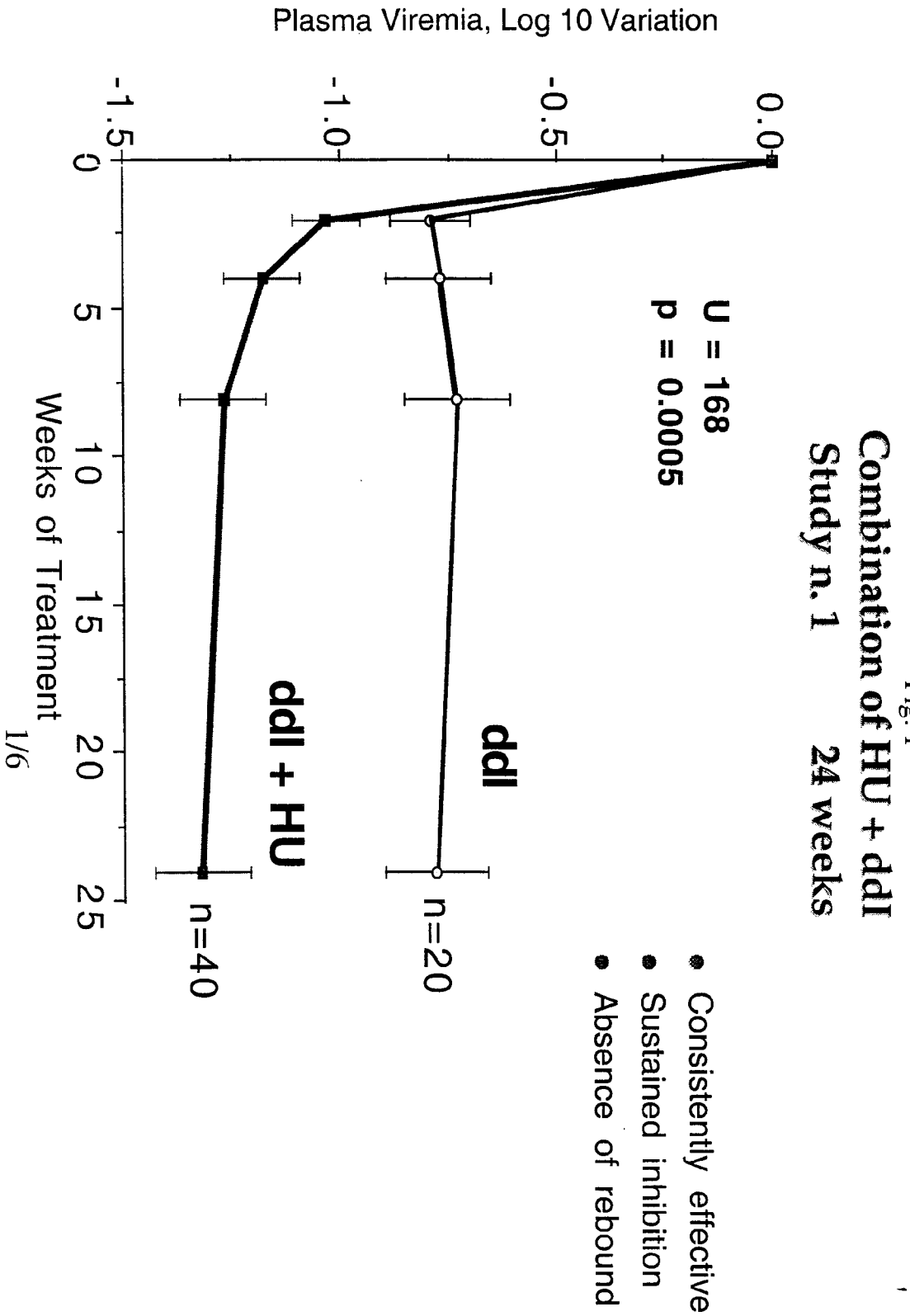
4. The method of Claim 1, further comprising the step of administering during the course of treatment at least one agent for activating quiescent cells harboring the virus.

5. An improved method of treating HIV infection in human beings where the patient is failing other therapies, comprising the steps of :
testing the blood for viral load;
if the viral load is above about 50,000-100,000 copies per ml, administering a drug combination consisting of hydroxyurea, one or more reverse transcriptase inhibitors, and one or more protease inhibitors;
if the viral load is below about 50,000-100,000 copies per ml, administering a drug combination consisting of hydroxyurea and one or more reverse transcriptase inhibitors.

6. The method of Claim 5, wherein the reverse transcriptase inhibitor is selected from ddI, d4T, 3TC, AZT, delavirdine, abacavir, adefovir, nevirapine, efavirenz, and mixtures thereof.

7. The method of Claim 5, wherein the protease inhibitor is selected from Indinavir, saquinavir, ritonavir, Nelfinavir, GW141, and mixtures thereof.

8. The method of Claim 5, further comprising the step of administering during the course of treatment at least one agent for activating quiescent cells harboring the virus.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• Absence of rebound

Fig. 1

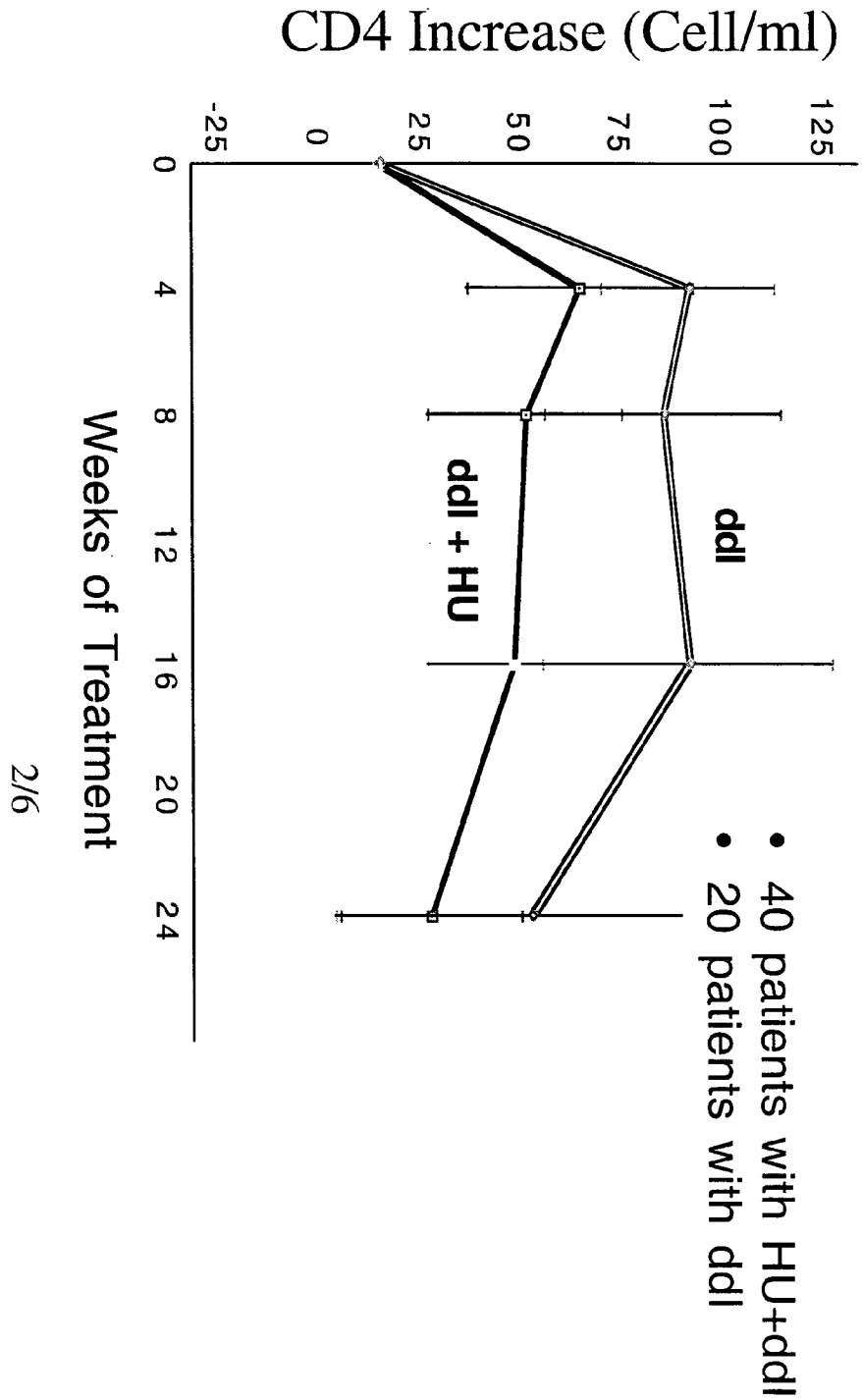
CD4 Increase (Cell/ml)
-25
0
25
50
75
100
125
0
4
8
12
16
20
24
Weeks of Treatment
ddI
ddI + HU
• 40 patients with HU+ddI
• 20 patients with ddI
CD4 Cell Count does not Increase
Significantly after HU+ddI Treatment

Fig. 1A

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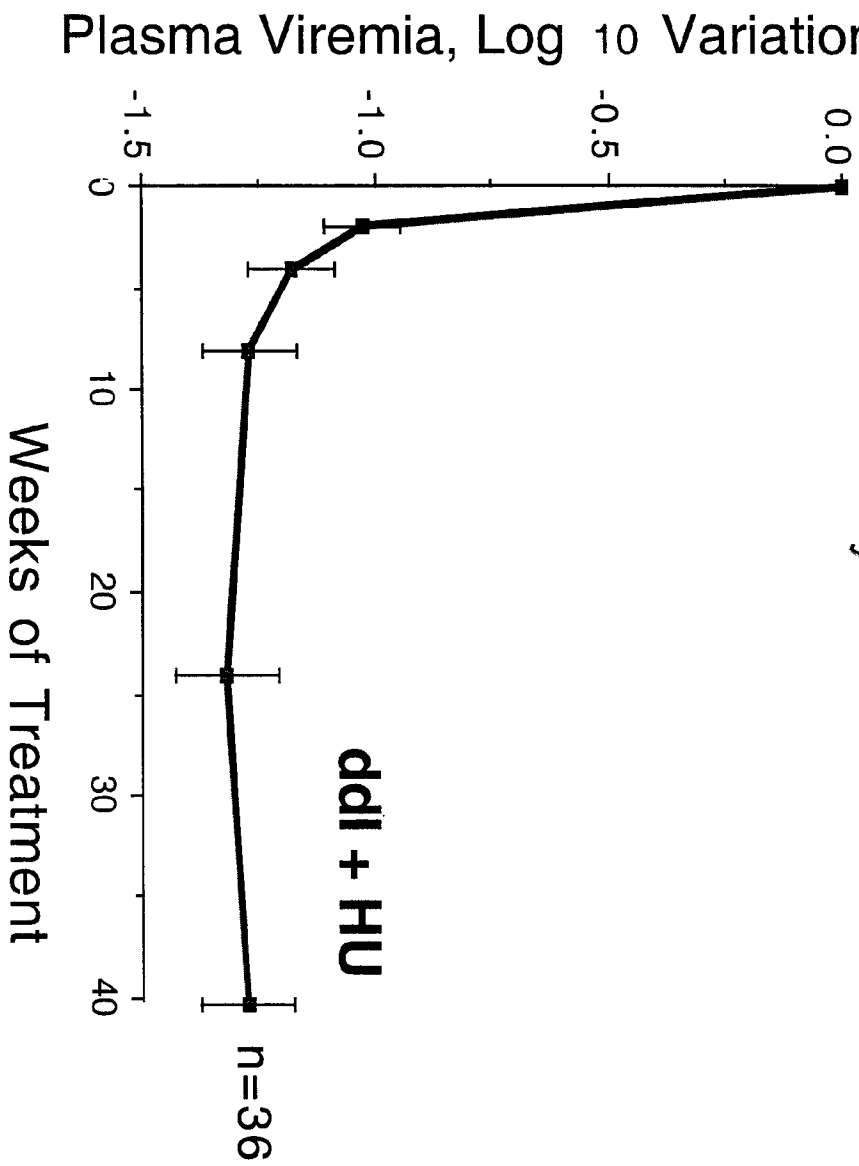

Fig. 2

Combination of HU + ddI
Study n. 1 40 weeks

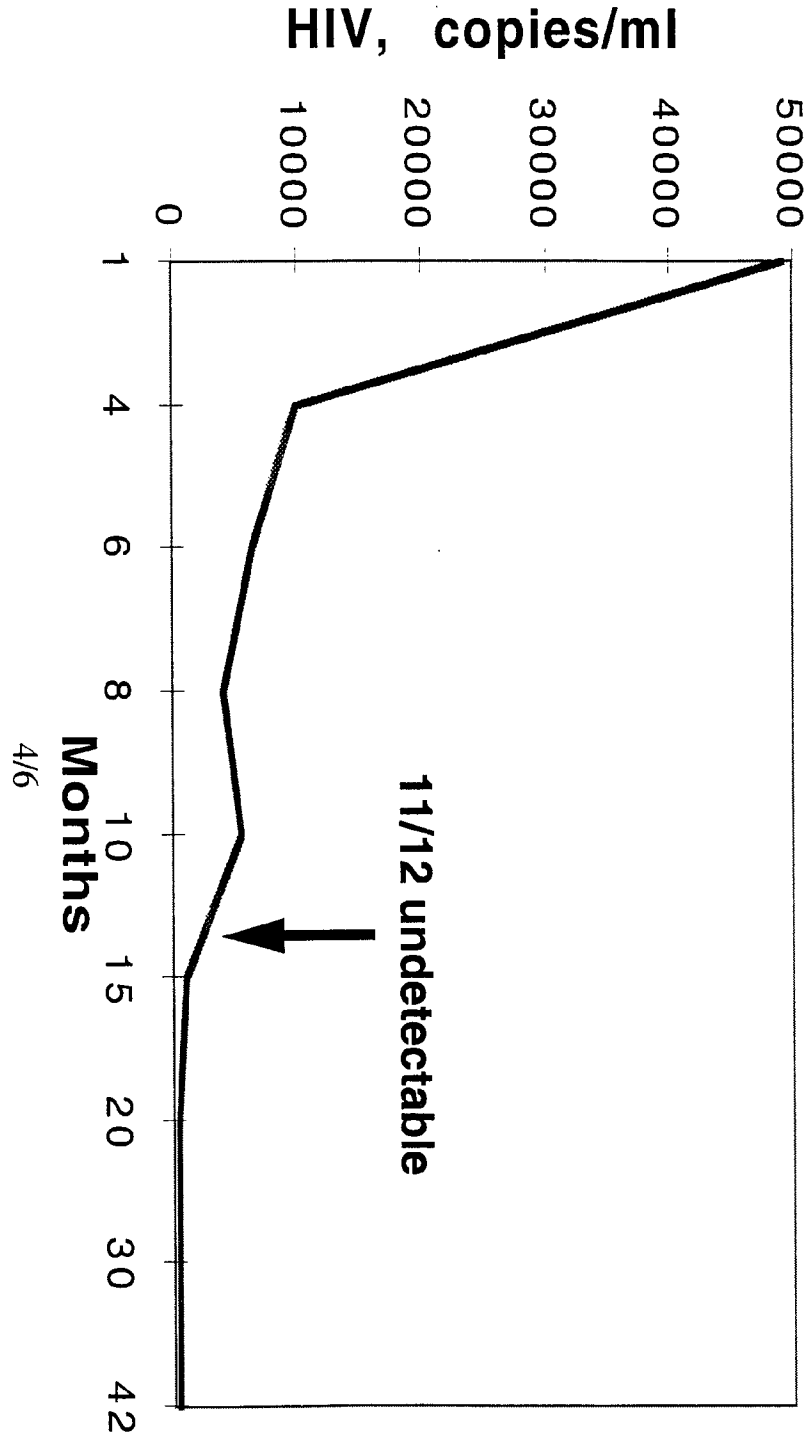
Plasma Viremia after Long-Term HU+ddI Therapy
12 Patients Follow-up (Average 28 months)
HIV, copies/ml
0
10000
20000
30000
40000
50000
1
4
6
8
10
15
20
30
42
Months
11/12 undetectable
4/6

Fig. 3

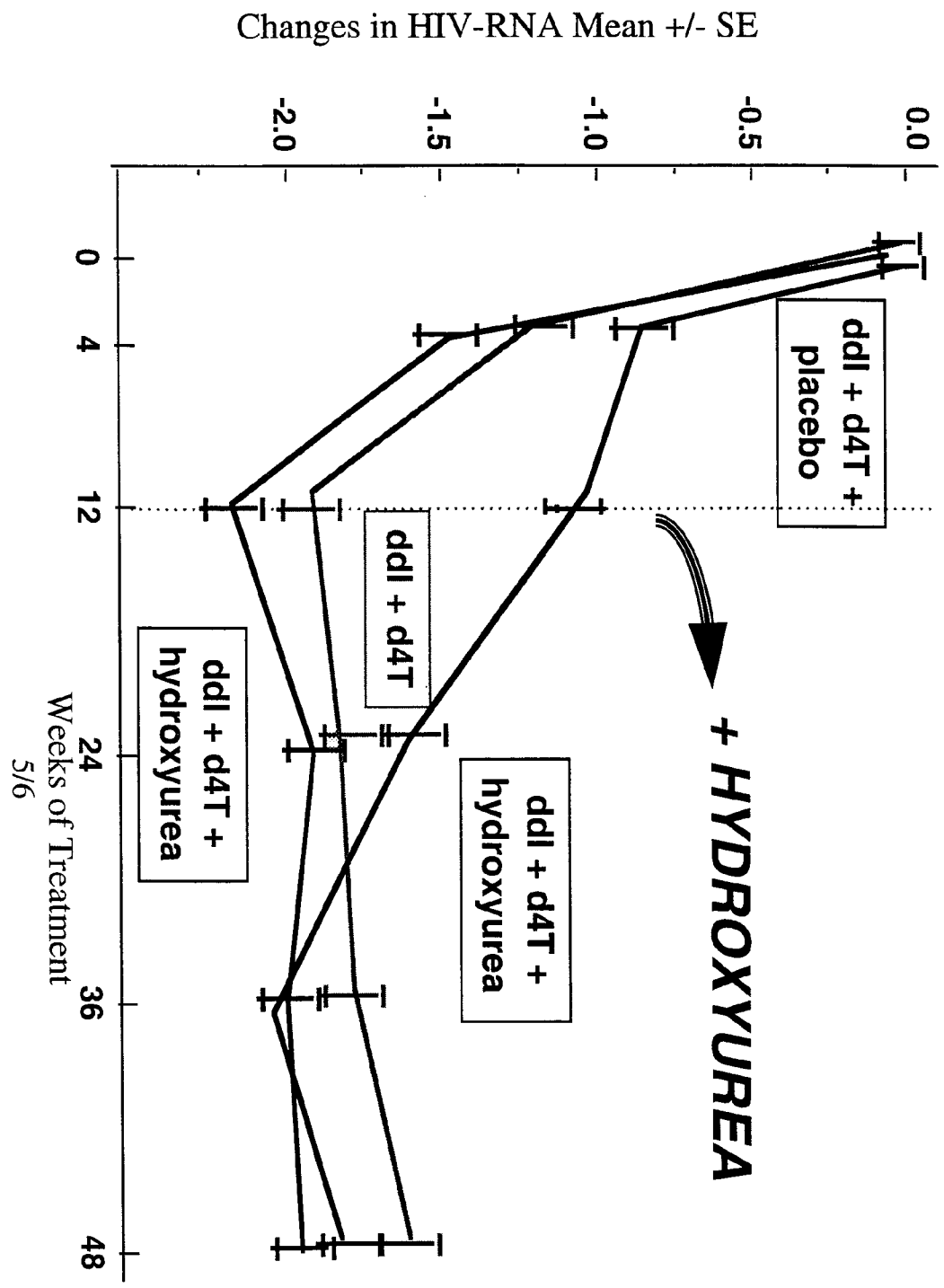

Fig. 4

ddH: viremia at 24, 36 and 48 Weeks

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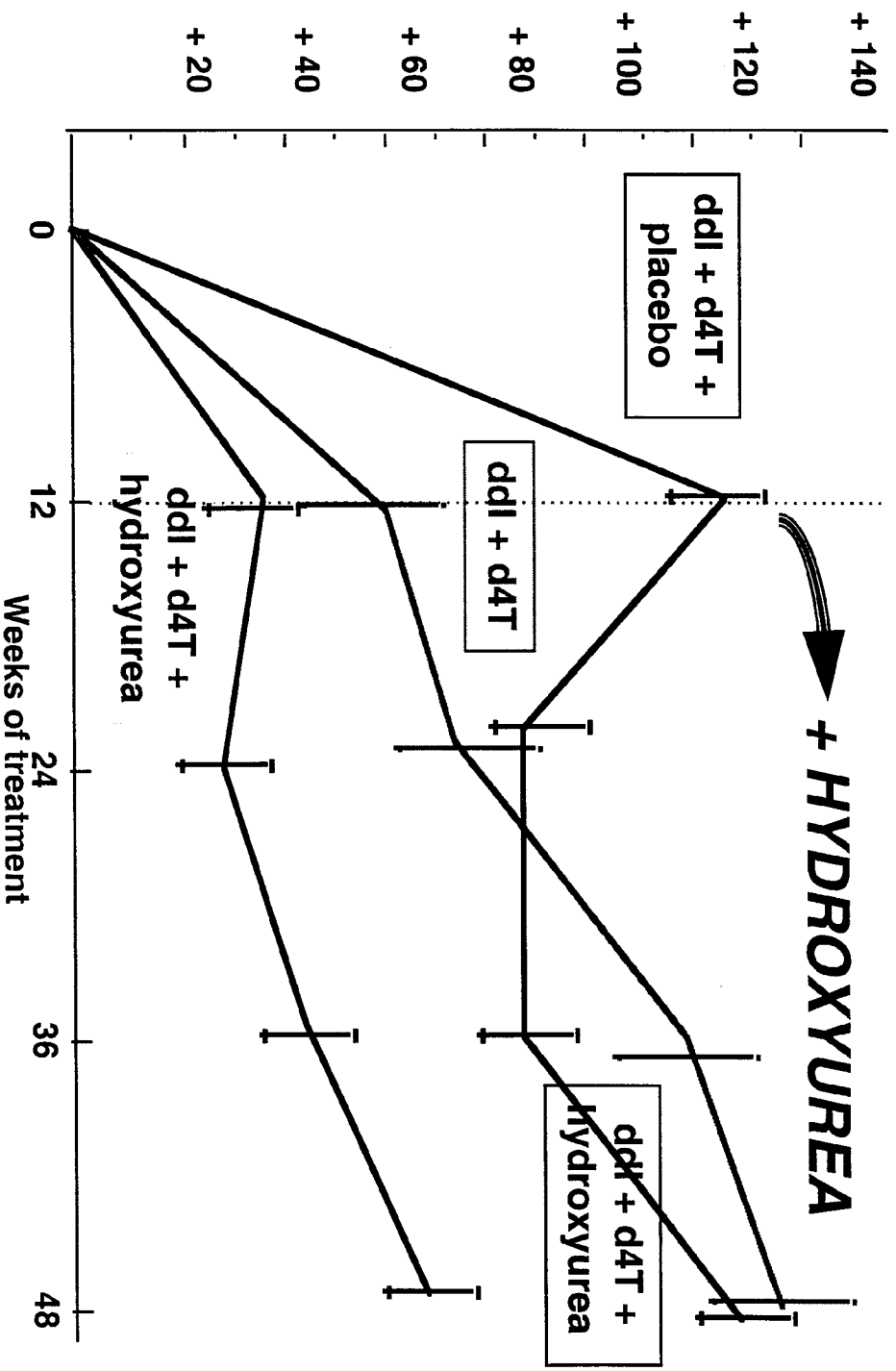

Fig. 4A

ddH: CD4 at weeks 24, 36 and 48

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INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/US 99/03452

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/70 //(A61K31/70,31:495,31:17)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Drug used for 30 years to battle cancer enlisted in war on AIDS." BIOTECHNOL. NEWSWATCH, 7, 16 February 1998 (1998-02-16), XP002112415 page 7, column 12, line 1-6 --- -/--	1-3,5-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 1999	01/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Leherte, C

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INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 99/03452

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE AIDSLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM),BETHESDA, MD, US LORI F ET AL: "Consistent, sustained HIV suppression without rebound by hydroxyurea, ddI and a protease inhibitor prevents loss of immunologic functions." retrieved from DIALOG Database accession no. 00184935 XP002086711 abstract & 5TH CONF RETROVIR OPPOR INFECT, FEB 1-5 1998, P203 (ABSTRACT NO. 655), ---	1-3,5-7
X	GILDEN D ET AL: "Dethroning AZT." GMHC TREAT ISSUES, FEB 1997, 11 (2) P10-3, XP002048480 page 12, column 1, line 4 - column 2, paragraph 5 ---	1,2,5,6
X	PALMER, SARAH ET AL: "Increased activation of the combination of 3'-azido-3'-deoxythymidine and 2'-deoxy-3'-thiacytidine in the presence of hydroxyurea" ANTIMICROB. AGENTS CHEMOTHER., FEB 1997, 41, 460-464, XP002048481 page 463, column 1, paragraph 2 ---	1,2,5,6
X	DATABASE AIDSLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM),BETHESDA, MD, US ROSSERO R ET AL: "Open label combination therapy with stavudine, didanosine, and hydroxyurea in nucleoside experienced HIV-1 patients." XP002048482 abstract & 4TH CONF RETRO AND OPPORTUN INFECT, JAN 22-26 1997, P166 (ABSTRACT NO. 549), ---	1,2,5,6
X	DATABASE AIDSLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM),BETHESDA, MD, US RUTSCHMANN OT ET AL: "ddI + d4T +/- hydroxyurea in moderately immunosuppressed HIV-1 infected patients." XP002048483 abstract & 4TH CONF RETRO AND OPPORTUN INFECT, JAN 22-26 1997, P166 (ABSTRACT NO. 550), --- -/--	1,2,5,6

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INTERNATIONAL SEARCH REPORT

Im tional Application No
PCT/US 99/03452

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE AIDSLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM),BETHESDA, MD, US LORI F ET AL: "Overcoming drug resistance to HIV-1 by the combination of cell and virus targeting." XP002048484 abstract & 4TH CONF RETRO AND OPPORTUN INFECT, JAN 22-26 1997, P173 (ABSTRACT NO. 589), ---	1,2,5,6
X	WO 94 27590 A (US HEALTH) 8 December 1994 (1994-12-08) claims 1-12 ---	1,2,5,6
X	VILLA ET AL: "Absence of viral rebound after treatment of HIV-infected patients with didanoside and hydroxycarbamide" LANCET THE, vol. 350, 30 August 1997 (1997-08-30), page 635/636 XP002086710 ISSN: 0140-6736 the whole document ---	1,2,5,6
X	WO 96 28162 A (UNIV MEDICINE & DENTISTRY OF N) 19 September 1996 (1996-09-19) claims ---	1,2,5,6
X	WO 96 22777 A (AGUETTANT LAB) 1 August 1996 (1996-08-01) claims ---	1,2,5,6
X	WO 95 17899 A (AGUETTANT LAB ;VILA JORGE R (FR)) 6 July 1995 (1995-07-06) claims -----	1,2,5,6

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 03452

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-8
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 /03452

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims relate to an extremely large number of possible combinations. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compositions claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed by the examples mentioned in the description at pages 11-12

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 99/03452

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9427590 A	08-12-1994	AU 685128 B	15-01-1998
		AU 6951994 A	20-12-1994
		CA 2163456 A	08-12-1994
		EP 0706387 A	17-04-1996
		JP 8509957 T	22-10-1996
WO 9628162 A	19-09-1996	NONE	
WO 9622777 A	01-08-1996	AU 4398296 A	14-08-1996
		US 5736526 A	07-04-1998
WO 9517899 A	06-07-1995	US 5521161 A	28-05-1996
		AU 1133899 A	04-03-1999
		AU 1821295 A	17-07-1995
		CA 2179627 A	06-07-1995
		EP 0735890 A	09-10-1996
		US 5736526 A	07-04-1998
		US 5736527 A	07-04-1998
		ZA 9409219 A	01-08-1995